Physiology of the Graafian Follicle and Ovulation

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Contents

The plate sections are between pp. 140 and 141, and pp. 268 and 269.

List of abbreviations

ACTH	adrenocorticotrophic hormone
ANOVA	analysis of variance
bFGF	basic fibroblast growth factor (FGF-2)
BMP	bone morphogenetic protein
cAMP	cyclic 3′,5′-adenosine monophosphate (cyclic AMP)
CGRP	calcitonin gene-related peptide
CSF	(1) cytostatic factor; (2) colony stimulating factor
CTP	carboxy-terminal peptide
Cx43	connexin 43
E-cadherin	epithelial cadherin
eCG	equine chorionic gonadotrophin
EGF	epidermal growth factor
EGFR	EGF receptor
eNOS	endothelial NOS
FADD	Fas-associated protein with death domain
FasL	Fas ligand
α-FF antigen	α-follicular fluid antigen
FF-MAS	follicular fluid MAS
FGF	fibroblast growth factor
FGIF	follicle growth inhibitory factor
FLICE	FADD-like IL-1β-converting enzyme
FLIP	FLICE/caspase 8 inhibitory protein
FSH	follicle stimulating hormone
GAG	glycosaminoglycan
GCSF	granulocyte colony stimulating factor
GDF	growth differentiation factor
GMCSF	granulocyte–macrophage CSF
GnRH	gonadotrophin releasing hormone

GnSAF	gonadotrophin surge attenuating factor
GnSIF	gonadotrophin surge inhibiting factor
GRP	gastrin releasing peptide
HB-EGF	heparin-binding epidermal growth factor
hCG	human chorionic gonadotrophin
HGF	hepatocyte growth factor
hMG	human menopausal gonadotrophin
hPG	human pituitary gonadotrophin
IFN	interferon (e.g. IFN-γ)
Ig	immunoglobulin
IGF	insulin-like growth factor
IGF-1R	IGF-1 receptor
IGFBP	IGF-binding protein
IL	interleukin (e.g. IL-1)
IL-6/LIF	interleukin 6/leukaemia inhibitory factor
iNOS	inducible NOS
i.u.	international unit(s)
IVF	in vitro fertilisation
LH	luteinising hormone
LHRH	LH releasing hormone
LIF	leukaemia inhibitory factor
M	monocyte
MAP kinase	mitogen activated protein kinase
MAS	meiosis activating substance/sterol
MCP	monocyte chemoattractant protein
MCSF	macrophage CSF
MIS	(1) meiosis-inducing substance; (2) Müllerian inhibiting substance
MMP	matrix metalloprotease
MPF	metaphase promoting factor
MPGF	male pronucleus growth factor
MPS	meiosis-preventing substance
M_r	relative molecular mass
MRNA	messenger RNA
N-cadherin	neuronal cadherin
NCAM	neural cell adhesion molecule
NE	norepinephrine
NG	neutrophilic granulocyte
NGF	nerve growth factor
nNOS	neuronal NOS

NOS	nitric oxide synthase
NPY	neuropeptide Y
OHSS	ovarian hyperstimulation syndrome
PA	plasminogen activator
PAF	platelet activating factor
PAPP-A	pregnancy associated plasma protein A
$P\text{CO}_2$	partial pressure of carbon dioxide
PCOS	polycystic ovarian syndrome
PDGF	platelet-derived growth factor
PG	prostaglandin (e.g. $\text{PGF}_{2\alpha}$)
PGS	prostaglandin synthase
PKA	protein kinase A
PMSG	pregnant mare serum gonadotrophin
$P\text{O}_2$	partial pressure of oxygen
PP5	placental protein 5
rFSH	recombinant FSH
rLH	recombinant LH
ROS	reactive oxygen species
SCF	stem cell factor (Steel factor)
SDS-PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SHBG	sex hormone-binding globulin
StAR	steroid or steroidogenic acute regulatory protein
TGF	transforming growth factor
TIMPs	tissue inhibitors of metalloproteases
TNF	tumour necrosis factor
tPA/uPA	tissue PA/urokinase PA
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal peptide
ZP	zona protein

I

Mammalian ovaries, Graafian follicles and oocytes: selected historical landmarks

Introduction

To the younger readers of this volume, the inclusion of a brief history of observations on mammalian ovaries may seem quite unnecessary, perhaps even an indulgence. Many such readers will have a molecular orientation, will be seeking a balanced assessment of recent research in their own highly specialised ovarian field, and will doubtless be hoping for inspiration and fruitful new lines of enquiry. All this is readily appreciated, as is the fact that most of the younger generation will have had neither the time nor the inclination to browse in the libraries of an ancient university or medical school. Such activity is, of course, not to everyone's taste but it could be to the advantage of many, in particular to reflect on how their chosen field has developed down the decades or even centuries and to note the considerable contributions of their predecessors. Viewed on such an extended timescale, their own sophisticated researches on a new growth factor, binding protein, gene sequence or mutation may fall into a different perspective: excellent – even distinguished – work certainly, but overall only a tiny fraction of the jigsaw that constitutes an understanding of ovarian function in the year 2002. So absolutely no apology is offered for the concise chapter that follows. Rather, the wish is expressed that all those who handle this volume will spare a few moments to glance at some of the studies undertaken before they themselves came on the scene. A valuable history on discovery of the ovaries can be found in Short (1977).

Steps in classical antiquity

Although its component parts and their relationships were far from being appreciated, the reproductive system attracted the attention of ancient Greek philosophers and physicians, the female organs in particular becoming the subject of

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classical works. Here, most attention appears to have focused on the uterus, even though the ovaries would undoubtedly have been seen by Hippocrates (460–370 B.C.). They came to be thought of as the 'female testes', but this was not the view of Aristotle (384–322 B.C.). He regarded the horned (bifurcated) uterus as representing the female gonads, and within this scheme he interpreted the *catamenia* (i.e. menstrual coagulum) elaborated by the uterus as the female contribution to generation of an embryo. Intermingling of the female fluid with the male semen or *sperma* in some remarkable manner formed the rudiment of the fetus – the heart – within the uterus. The heart then directed elaboration of a complete fetus from further menstrual blood. In this concept, there was clearly no requirement to highlight the ovaries, even though Aristotle must have examined these prominent structures during his extensive observations on many species of animal and doubtless pondered their significance.

Within this Western – essentially Mediterranean – tradition, credit for first specifically describing human ovaries is generally given to Herophilus of Chalcedon, a third century B.C. anatomist who taught and practised medicine at Alexandria. His major treatise on midwifery came to be highly esteemed and widely consulted during antiquity. Despite such acclaim, he had introduced an erroneous line of thought, stemming from poor observation and considerable imagination. Even though he described the uterus and cervix, Herophilus presumed that the ducts now termed Fallopian tubes transmitted female semen from the ovaries (the 'female testes') to the urinary bladder. To propose a generative rôle for the ovaries was clearly inspirational, and a bold step in that it disagreed with Aristotle's view of the uterus as the source of female semen. Even so, the failure of Herophilus to have followed the Fallopian tubes to their correct destination appears surprisingly careless to modern eyes, and transmission of female semen to the bladder would seem unusual in any scheme of reproduction.

Soranus of Ephesus (*circa* A.D. 100), a Greek physician who practised medicine in Rome, perpetuated the misinterpretation that ducts pass from the ovaries to the urinary bladder in his classical work on gynaecology. Having studied in Alexandria and been much influenced by the descriptions of Herophilus, Soranus was in effect re-stating that the putative female seed has no concern with procreation, being expelled from the body with the urine. The extensive writings of Soranus had a prominent influence on the Latin West, and the views assembled in his *Gynaecia* long outlived him, indeed in some quarters for many centuries (Temkin, 1956). In a context of a rôle for the ovaries, and in the light of subsequent contributions by Galen, this may come as a considerable surprise. However, clear evidence has been offered by Bodemer (1969) for the manner in which sixteenth century texts were influenced by the views of Soranus.

The Greek physician and biologist, Galen (A.D. 130–200) also followed the traditional pilgrimage across the Mediterranean to the renowned medical school at Alexandria. He studied there from A.D. 152 to A.D. 157 before progressing to Rome and, although strongly taken by the writings of Herophilus, he escaped from prevailing doctrines in proposing that the female does elaborate semen by filtration from the bloodstream within the ovaries ('female testicles'). Such semen would then be carried through the Fallopian tubes, which, he correctly noted, coursed to the uterus and not to the bladder. Despite this anatomical evidence based in part on observations of the bicornuate uterus in domestic ungulates, Galen envisaged that it was the male and female fluids intermingling within the uterus that coagulated to form a fetus. His views thus restored some prominence to the ovaries in the process of generation and in one sense gave them parity with the male gonads. The doctrines of Galen with their echoes of Aristotle remained prominent for almost fourteen centuries after his death, undoubtedly a mark of his commanding stature in the medical world. In a reproductive context, such was the state of enquiry and of philosophical endeavour towards the end of the Graeco-Roman period – a legacy of ideas and speculation that remained prominent well into the period of mediaeval thought and teaching. Although the wonderful drawings of Leonardo da Vinci (1452–1519) took shape during the latter part of this span, many were lost and those remaining were not published systematically for the benefit of the scientific world until the late nineteenth century. For the historical record, however, it is worth noting that Leonardo clearly depicted the gravid human uterus and indeed the morphology of the neighbouring ovary (Fig. I.1). Moreover, generations of medical students owe a special debt to Leonardo in that he added excitement to the topic of placentation by bestowing the cotyledonary specialisations of the cow placenta upon the materno-fetal interface in humans. How did this come about? Simply that he supplemented his painstaking dissection of human cadavers with animal specimens, not least ones obtained from the slaughterhouse.

Sixteenth and seventeenth century contributions

As is nowadays quite widely appreciated, aesthetically pleasing and surprisingly accurate drawings of the human reproductive organs were published in 1543 in the masterpiece of Andreas Vesalius (1514–1564). Born in Brussels, he studied in Louvain and Paris and then held a professorship in the medical school of his adopted Padua. The seven volumes of his work were together entitled *De Humani Corporis Fabrica Libri Septem*, although they are usually referred to simply as the *Fabrica*. Generally considered to form the basis of
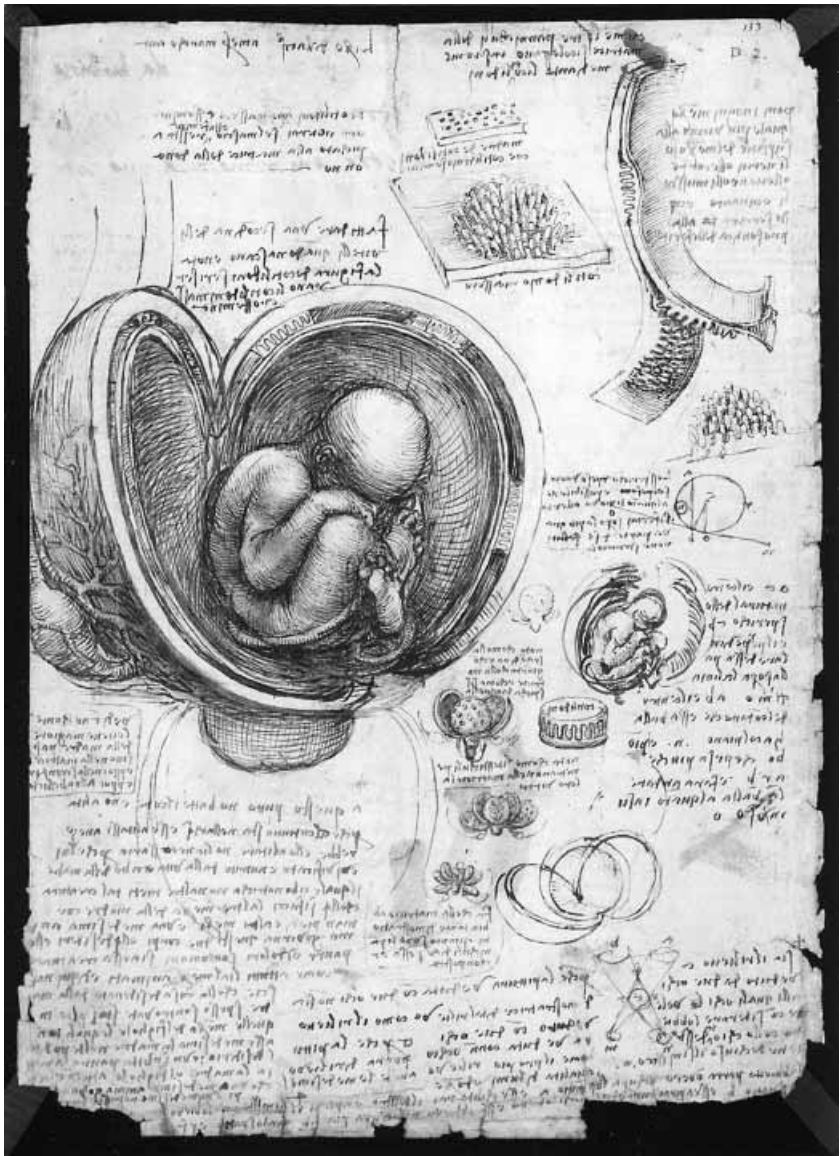
Fig. I.1. The wonderful illustration by Leonardo da Vinci of a gravid uterus, clearly depicting one of the ovaries and associated blood vessels.

As an aside, Leonardo has taken the remarkable liberty of introducing the cotyledonary placenta of a cow into a human uterus. One explanation offered for this error is that by the time the corpse was available for dissection, the contents of the uterus were in an advanced state of degeneration, so tissues were procured from a local abattoir to enable completion of the drawing. (Courtesy of 'The Royal Collection © 2001, Her Majesty Queen Elizabeth II'.)

modern systematic teaching and research in anatomy, the *Fabrica* illustrate the disposition of the ovaries and Fallopian tubes, but the interpretation of their relationship retains a strong flavour of Galen. Vesalius inferred that the ovaries and Fallopian tubes played a rôle parallel to that of the testes and associated male ducts. The tubes were thus interpreted as 'semen conveying' vessels and, with considerable artistic licence, illustrated in Book V of the *Fabrica* as ducts coiled around the 'female testes' (Fig. I.2). In fairness to Vesalius, it is worth emphasising that the existence of male and female gametes had not yet even entered the imagination, let alone the notion that the ovaries might be the source of egg cells (i.e. oocytes).

Fallopius (Gabriele Fallopio, 1523–1562), a sometime pupil of Vesalius who succeeded to the same Chair in Padua, did not take matters forward in the above regard. Although he produced the first specific description of the Fallopian tubes in his *Observationes Anatomicae* (1561), he still regarded these structures as seminal ducts for the transmission of female semen. In other words, a misinterpretation coupled with a too-willing acceptance of dogma handed down through the ages. Little could Fallopius have imagined that these 'uterine tubes' would one day be shown to provide the meeting place of the gametes, that is, the actual site of the process of fertilisation in mammals. The uterus was still regarded as the organ wherein the embryo was generated and yet – as one notes with hindsight – Fallopius provides a wonderfully eloquent description of the fimbriated extremity of the tube, the portion that actually 'captures' the egg from the ovary at ovulation and displaces it into the ostium of the Fallopian tube.

> This seminal duct (*meatus seminarius*) originates from the cornua uteri; it is thin, very narrow, of white colour and looks like a nerve. After a short distance it begins to broaden and to coil like a tendril (*capreolus*), winding in folds almost up to the end. There, having become very broad, it shows an *extremitas* of nature of skin and colour of flesh, the utmost end being very ragged and crushed, like the fringe of worn out clothes. Further, it has a great hole which is held closed by the fimbriae which lap over each other. However, if they spread out and dilate, they create a kind of opening which looks like the flaring bell of a brazen tube. Because the course of the seminal duct, from its origin up to its end, resembles the shape of this classical instrument – anyhow, whether the curves are existing or not – I named it *tuba uteri*. These uterine tubes are alike not only in men, but also in the cadavers of sheeps and cows, and all the other animals which I dissected.
>
> *(Herrlinger & Feiner, 1964)*

Similarly attracted to the Padua school was a pupil of Fallopius called Fabricius (1537–1619), who in due course also succeeded to the Chair of Anatomy. He concluded that the Fallopian tube or duct was an organ of secretion, having produced a reasonably accurate account of its function in the formation
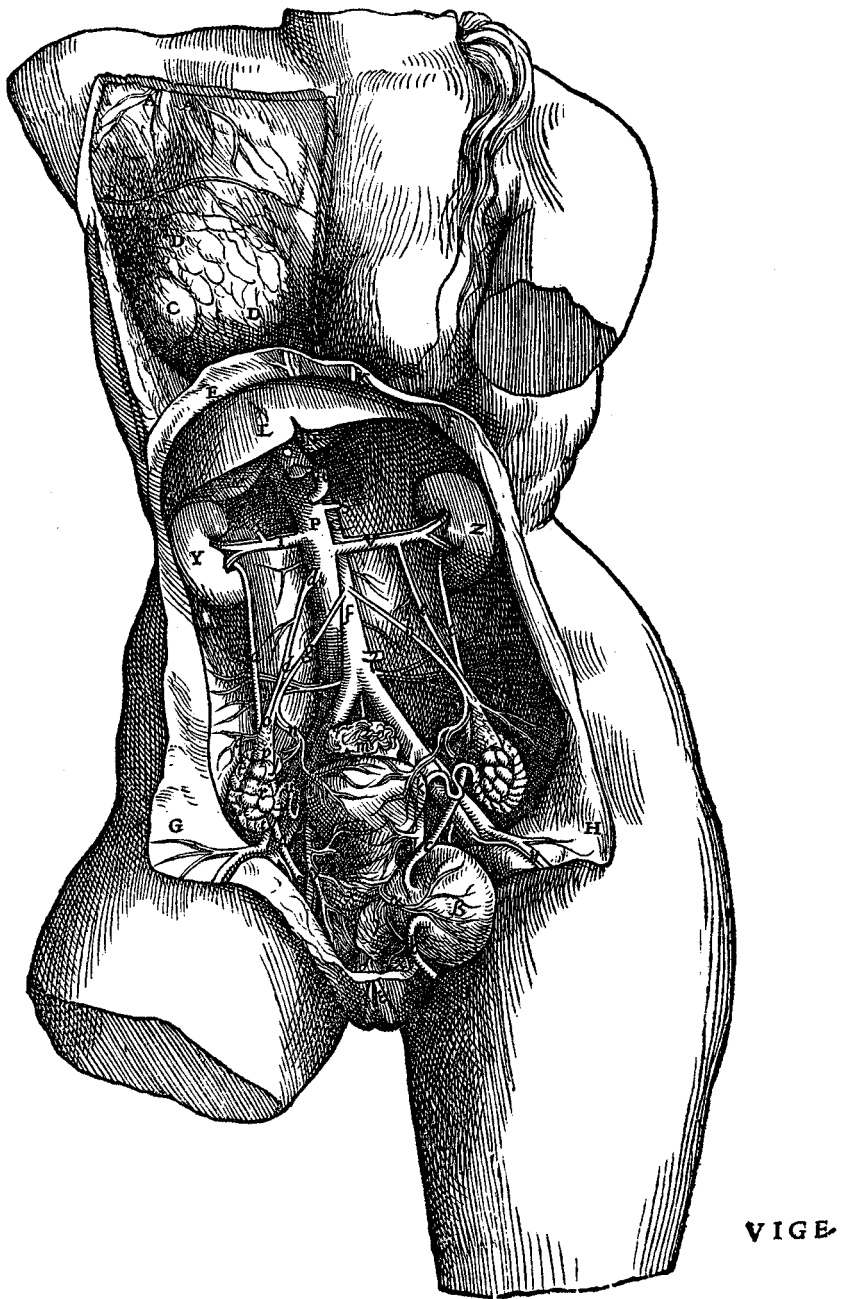
Fig. I.2. To specialists in mammalian reproduction, this is perhaps the most appealing plate from the *Fabrica* of Andreas Vesalius (1543). Both ovaries feature prominently, even though seemingly embraced by coiled ducts at the extremity of the Fallopian tubes. Such an arrangement would correspond to the disposition of epididymal tissues alongside the male gonad. (Courtesy of Glasgow University Library.)

of chicken eggs. As a gifted anatomist, Fabricius provided accurate descriptions of the uterus and ovaries, and likewise of the uterine and ovarian arteries and their anastomoses. Indeed, in a splendid drawing from *De Formato Foetu* (1604), depiction of the ovaries alongside the gravid uterus of a sow is sufficiently precise to enable individual corpora lutea to be distinguished (Fig. I.3). Overall, however, and despite his contributions on the chicken egg and on embryology of the chick, Fabricius did not abandon completely the theories of Galen concerning the involvement of semen and menstrual blood in mammalian generation.

As is well known in both medical and historical circles, the Englishman William Harvey (1578–1657) trained in Cambridge and then completed his formal education at the University of Padua (1602), undoubtedly still the leading medical school in Europe. He was there a sometime student of Fabricius and much interested in reproduction – a sphere quite distinct from that in which he subsequently achieved fame, i.e. circulation of the blood. Harvey examined the contents of the uterus in various species of mammal after they had mated and, largely on the basis of his studies in red and fallow deer in which no trace of conception could be found for a protracted interval after the onset of rutting, concluded that the existence of female semen was a myth and indeed that male semen was not involved in formation of the fetus. He was thus out of step with his distinguished predecessors, the more so since he proposed a new theory of generation far removed from concoctions of semen and menstrual blood that had featured since antiquity. Although Harvey in no sense suggested a specific function for the mammalian ovaries, nor did he even entertain a rôle for them in reproduction, he was sufficiently inspired by his work on the chicken embryo to propose that: *Ex ovo omnia* – 'All living things come from eggs' (Fig. I.4) in his *Exercitationes de Generatione Animalium* (1651). Actual evidence in support of this magisterial statement became available only some 200 years later in the reports of Barry (1843), Bischoff (1854) and Van Beneden (1875) on sperm penetration into the mammalian egg. Nonetheless, Harvey did think in terms of epigenesis – a gradual emergence of the differentiating embryo and fetus from the egg – rather than endorsing the concept of preformation. In error, he considered that the embryo itself was generated from a drop of blood that is elaborated within the fertile egg.

Conducting studies in Leiden, where he was Professor of Anatomy, van Horne (1668) clearly distinguished ovarian vesicles or follicles, but thought of them as eggs and imagined that the Fallopian tubes might in some manner function to transmit the vesicles to the uterus. Initially working with avian gonads (Fig. I.5), it was Regnier de Graaf (1641–1673) of Delft (see Frontispiece) who highlighted the tertiary or vesicular follicles that were later to take his name
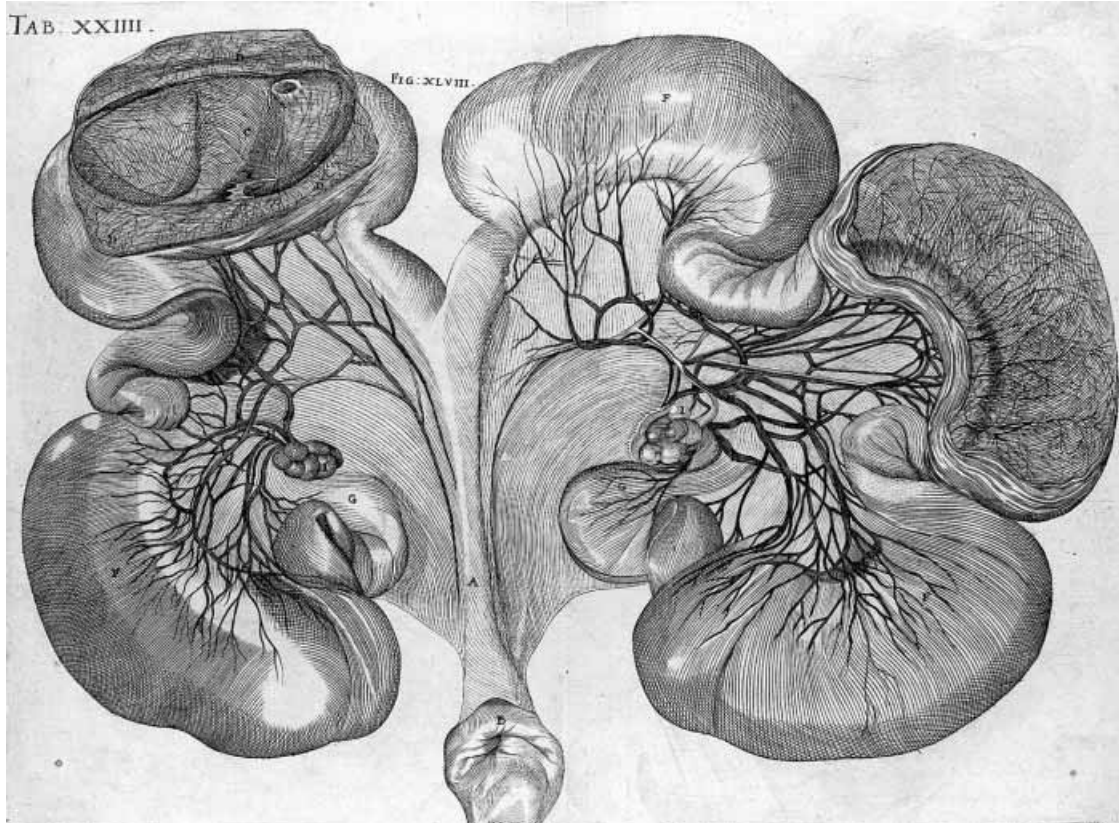
Fig. I.3. An extremely accurate illustration taken from *De Formato Foetu* (Fabricius, 1604) in which the bicornuate uterus of a pig is displayed at an advanced stage of gestation. Dominated by the presence of mature corpora lutea, the ovaries and associated blood vessels are clearly distinguishable. (Courtesy of Cambridge University Library.)

Fig. I.4. The splendid plate that appears as Frontispiece to William Harvey's *Exercitationes de Generatione Animalium* (1651). The caption states that 'All living things come from eggs', and Zeus is shown liberating live creatures from a substantial egg. (Courtesy of Edinburgh University Library.)
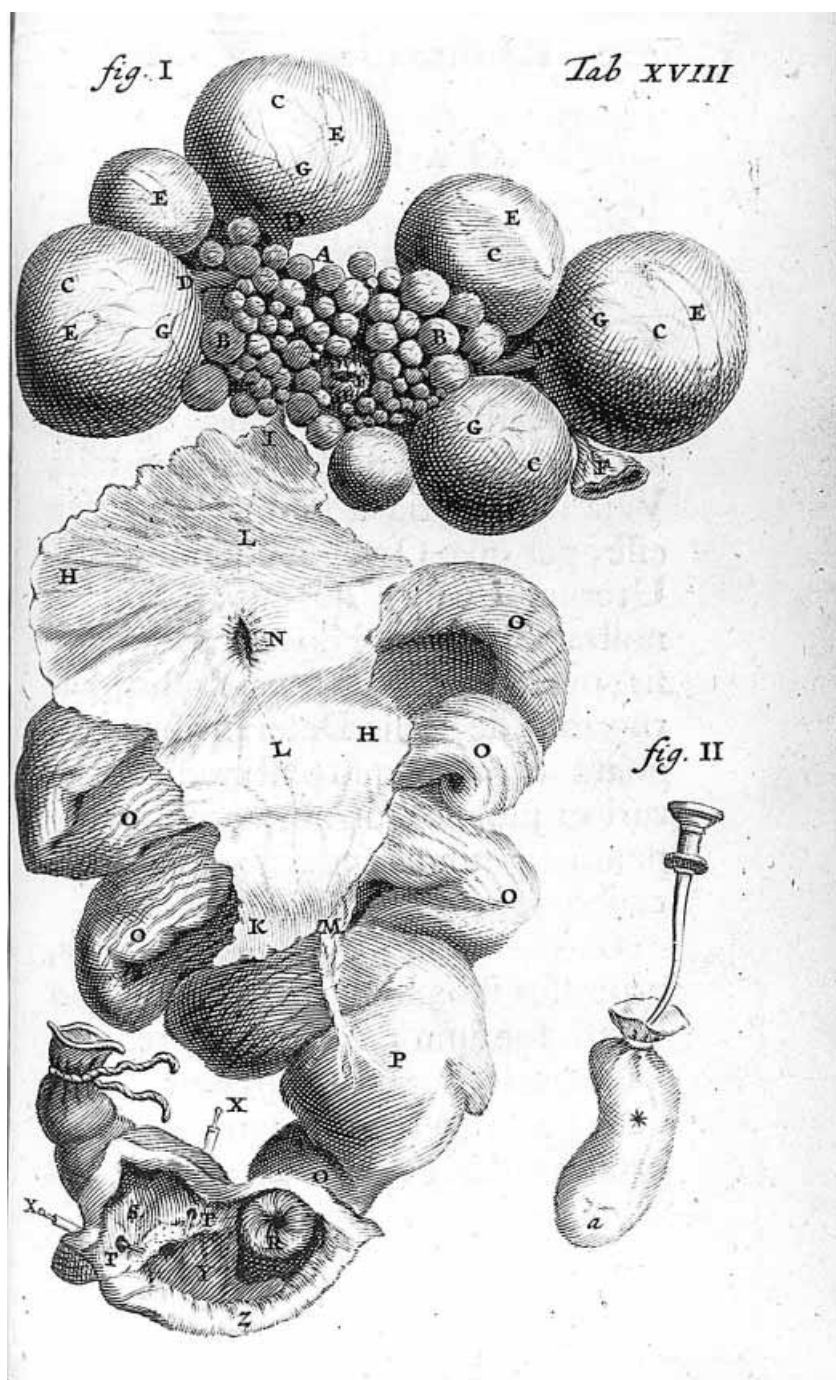
Fig. I.5. The ovary and adjacent duct system of a chicken as portrayed by Regnier de Graaf (1672). The ovarian follicles are much larger than the corresponding mammalian structures, for they become distended with yolk. (Courtesy of Edinburgh University Library.)

in mammalian ovaries. He maintained that the follicles were the source of the eggs, even though an egg transport function for the Fallopian tubes was not yet generally accepted: Harvey's (and Galen's) notion of conception – of an ovum formed *in utero* – still prevailed.

By 1672, however, de Graaf had traced the would-be passage of eggs through the tubes to the uterus and he had also emphasised that sterility invariably follows the castration of females, a fact already known to Aristotle from observations on domestic animals. Although de Graaf had an appreciation of ovarian function, his mistake was to believe that the follicles were eggs. Quite apart from his avian studies, he was here led astray by observations in the rabbit – an induced ovulator – in which the large follicles disappeared after copulation to be replaced by corpora lutea and yet were presumed to be the blastocysts noted in the uterus a few days later. As de Graaf himself fully appreciated, the problem of dimensions of the ovarian follicles in relation to the diameter of the Fallopian tubes could not be reconciled with the passage of eggs unless, in reality, it was the contents of the follicles that entered the tubes. But he had not observed actual rupture of the follicle or release of the egg, for microscopy was at its inception and de Graaf seems not to have been tempted by the primitive models then available to examine the contents of ovarian follicles. His drawings remain essentially large scale and those of human ovaries, Fallopian tubes and associated vasculature are particularly fine (Fig. I.6). As a postscript appropriate to mention at this point, de Graaf's recording of a tubal ectopic pregnancy (Fig. I.7) and his detailed discussion of this condition were used as a powerful argument in support of the proposition that the eggs from which fetuses are to be generated pass from the ovaries ('female testicles') to the uterus by way of the Fallopian tubes.

Before the close of the seventeenth century, there was a related – albeit posthumous – contribution from the famous Italian anatomist Marcello Malpighi (1628–1694). Malpighi had made his first known drawing of an ovary in 1666, a monkey ovary, and had clearly noted Graafian follicles. Apparently, he had reasoned that the actual egg must lie within the ovary and that the follicle itself was not shed from the ovary, perhaps as a result of his well-known correspondence with de Graaf. In contrast to de Graaf's original notion that the follicle itself was the egg, Malpighi proposed in 1681 that the egg was derived from the luteal tissue that was frequently present in a mature mammalian ovary (Adelmann, 1966). Working with cow ovaries, he recorded that the yellow tissue of the corpus luteum (his terminology) possibly contributed to formation of the egg, doubtless being led astray by analogies with yolk colour in the chicken egg, which he had studied intensively. To the extent that the corpus luteum arises from a Graafian follicle after ovulation, Malpighi had offered a
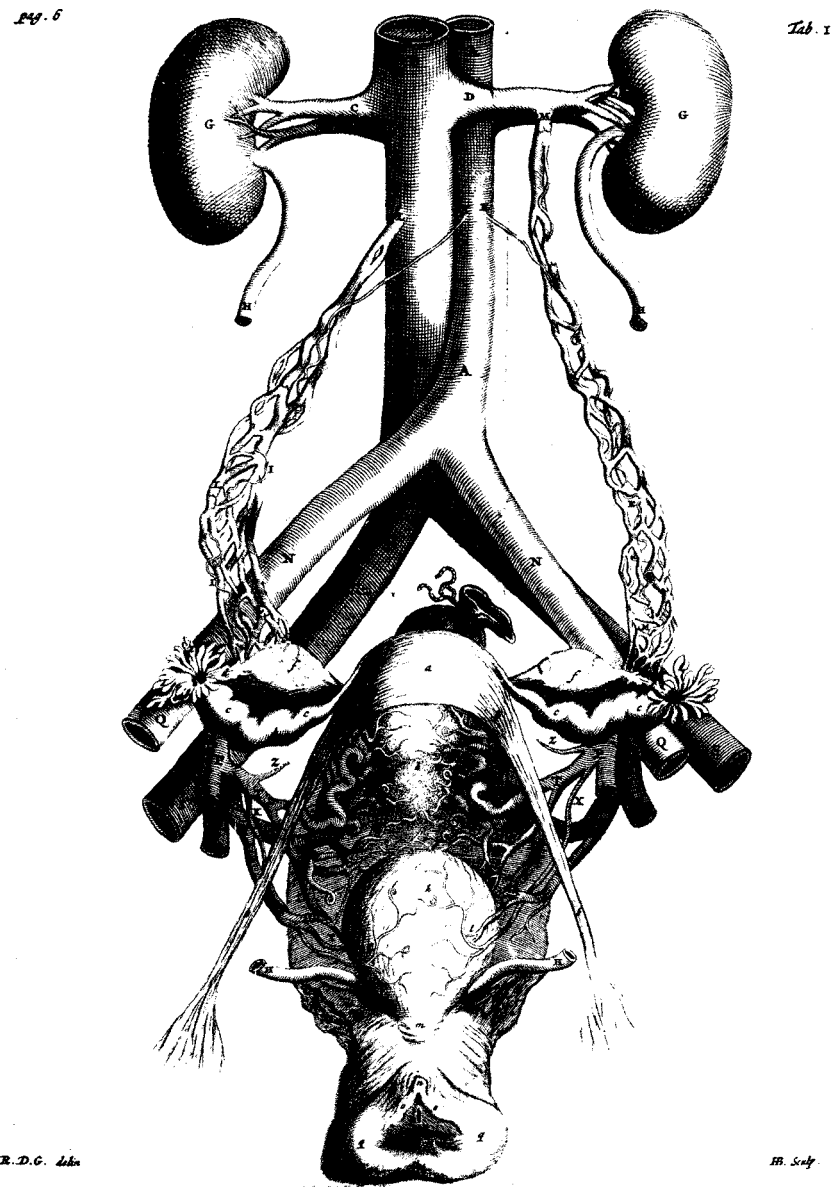
Fig. I.6. A drawing of the human uterus and Fallopian tubes as presented by Regnier de Graaf (1672). Both ovaries feature prominently, as do the major blood vessels supplying these vital organs. (Courtesy of Edinburgh University Library.)
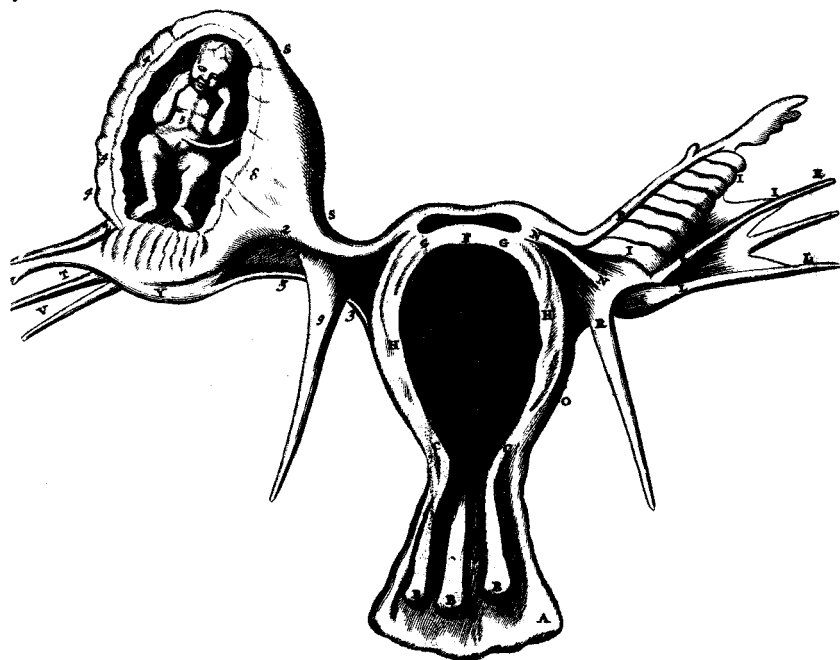
Fig. I.7. A tubal ectopic pregnancy as portrayed with some artistic licence in Regnier de Graaf (1672). Such an observation enabled de Graaf to deduce that eggs pass from the ovaries to the uterus through the Fallopian tubes. He added the somewhat macabre comment that 'as such a fetus grows, it prepares death for its mother'. (Courtesy of Edinburgh University Library.)

somewhat tenuous connection between the egg and a Graafian follicle. On the basis of only this link and the fact that luteinisation commences before ovulation, enthusiastic scholars of the Malpighi tradition have seemingly given their master undue prominence in discovering the origin of the mammalian egg. An elegant and convincing demonstration of this feature of the ovaries was almost 150 years into the future.

Soon after the two key publications of Regnier de Graaf (1668, 1672), translated by Jocelyn & Setchell (1972) and reviewed by Setchell (1974), microscopy began to shed light on the first steps of reproduction, although of course the significance of these earliest observations could not be clearly grasped. Another Dutchman, Antonie van Leeuwenhoek (1678), also of Delft, examined semen under his own rudimentary model of microscope and reported on the millions of little animalcules so observed to the Royal Society of London. This key stage was achieved in 1677 but only published in 1678 due to a delay in translation

from Dutch into Latin (Leeuwenhoek, 1678). The observations were made on the semen of fish, frogs and mammals and were thought to have constituted the first factual description of spermatozoa. Leeuwenhoek (1683) went on to postulate that de Graaf's 'egg' required to be impregnated by one of the animalcules for pregnancy to occur – an amazing piece of intuition – although no such observation was made at that time. As a postscript of some interest, it was de Graaf who introduced Leeuwenhoek to the Royal Society in 1673 (B.P. Setchell, personal communication).

Eighteenth and nineteenth century views

As improved models of microscope were developed, de Graaf's views and indeed findings were eventually endorsed in 1797 by William Cruikshank, who likewise identified rabbit eggs in the Fallopian tube on the third day after the animals had mated. It should be emphasised that this step was facilitated by the prominent mucin layer that accumulates around the eggs in this species during their passage towards the uterus. By 1827, von Baer had discovered the origin of the mammalian egg, and thereby had commenced to dispel the existing confusion between eggs and follicles. This was achieved by dissecting open Graafian follicles and examining the liberated contents within their cumulus masses. von Baer recorded the triumphant moment:

> Led by curiosity . . . I opened one of the follicles and took up the minute object on the point of my knife, finding that I could see it very distinctly and that it was surrounded by mucus. When I placed it under the microscope I was utterly astonished, for I saw an ovule just as I had already seen them in the tubes, and so clearly that a blind man could hardly deny it.
>
> *(Translation from Corner, 1933)*

At this time, von Baer was working in Königsberg but he was in fact born in Estonia and trained in medicine at Tartu University. As a sequel to this key publication concerning the origin of the mammalian ovum, there was frequent reference to 'Baer's bladder' (Syritsa & Kalm, 1999).

This was therefore the first significant step in understanding cellular components of the ovaries. In fact, Prévost & Dumas (1824) studying reproduction in the dog had anticipated von Baer's discovery in the sense that they had deduced that de Graaf's follicles probably contained the eggs and that fertilisation would occur only after shedding of such putative eggs from the ovary. They further speculated that perhaps fluid from the Graafian follicles assisted in transporting eggs to the uterus, although this is now appreciated not to be so, for the eggs or

embryos of most mammals so far examined remain in the Fallopian tubes for 2–3 days or more before passing through the utero-tubal junction. Follicular fluid entering the Fallopian tube at ovulation is refluxed rather promptly into the peritoneal cavity.

Bischoff (1842) suggested that contractions of the Fallopian tube and its mesenteries were necessary for passage of eggs to the uterus, and Thiry (1862) proposed that ciliary activity was responsible for the displacement of eggs from the ovaries into the tubes. Both of these advances followed Blundell's (1819) key work in which he drew attention to muscular movements of the Fallopian tube. Thus did physiological observations take tentatively to what had previously been overwhelmingly an anatomical stage. But not to be overlooked in this nineteenth century focus on eggs, their origin and displacement is the earlier classical experiment of John Hunter (1787) involving unilateral ovariectomy to deduce the contribution of individual ovaries to the long-term fertility of sows. Whereas Aristotle had a passing acquaintance with the behavioural influence of castration in sows, Hunter's work more than 2000 years later was the first exploitation of ovariectomy as a specific experimental approach to clarifying function. The study was far ahead of its time but imperfectly executed in the sense that the 'hemi-spayed' sow was slaughtered prematurely: its presumptive infertility was seemingly attributed to ovarian cysts, even though the animal periodically came into oestrus. Nonetheless, Hunter deduced that the ovary had a limiting finite power in terms of the number of offspring an animal can produce, a statement not to be faulted even today.

Surgical exploration of the reproductive organs was increasingly frequent in humans and animals during the nineteenth century, and laid the foundations of the discipline that would in due course become endocrinology of the twentieth century. The fact that menstruation was under ovarian control was gradually clarified (see Tilt, 1850; Corner, 1950), and corpora lutea slowly came to be appreciated as glands of secretion rather than as post-ovulatory scar tissue. Treatment with ovarian extracts was noted to produce morphological and be-havioural influences, and soon the uterus was seen as a target organ of ovarian activity, although not precisely in those terms. Grafting of ovaries into rabbits previously ovariectomised prevented atrophy of the uterine tissues (Knauer, 1896), indicating that the ovaries were glands of internal secretion – as they became called in due course. This step was quickly followed by the suggestion of Prenant (1898) that the corpus luteum itself was a gland of internal secretion, its product(s) entering directly into the bloodstream. Extracts of corpora lutea were soon prepared and diverse experiments undertaken to clarify the rôle of the supposed secretion.

Twentieth century highlights

Of the two major compartments of the ovaries after puberty (putting the stroma to one side), research seems to have concentrated more on the corpus luteum than on the Graafian follicle during the first part of the twentieth century. Numerous experiments were performed bearing on luteal function, in one sense culminating in the isolation of progesterone in the mid 1930s in George Corner's Rochester laboratory in the USA (Corner, 1942). As to the follicle, and after much confusion, a distinction was gradually drawn between spontaneous and induced ovulation, with some preliminary classification of species into the two categories. As may be imagined, there was considerable debate as to whether species thought to be spontaneous ovulators could become induced ovulators under appropriate conditions, a debate that continues to the present day – and in the reverse sense, too (see Chapter VIII). Bioassays were gradually developed for the secretory products of corpora lutea and Graafian follicles using selected target tissues, and such assays underwent progressive refinement during the first half of the century, eventually to be superseded by chemical, radio-chemical and even immunological assays. The sexual cycle of mammals had been defined in Heape's classical paper of 1900 and there then followed 40 years of intensive, exciting and even competitive endocrinology (see Parkes, 1962). Scientists in France, Germany, the Netherlands, Switzerland, the United Kingdom and the USA were all much involved. By now, in fact, there was vigorous participation of researchers at various North American universities, and not primarily those of the so-called Ivy League.

Not only were the ovarian steroid hormones isolated and analysed by the end of the 1930s, with synthesis of such steroids soon following, but it was appreciated by the mid 1920s that the ovaries themselves were under the control of the pituitary gland (Smith & Engle, 1927). Pursuit of the gonadotrophic hormones followed rapidly, initially by means of the skilled surgical intervention termed hypophysectomy. Separation of the lobes of the pituitary gland enabled gonadotrophic activity to be attributed to the anterior pituitary whereas the endocrine activity of the posterior pituitary, especially of the reproductive hormone oxytocin, was found to be derived from the hypothalamus. The concept of hypothalamic control of the anterior pituitary gland by means of peptide releasing factors was pursued most prominently and elegantly by G.W. Harris from the mid to late 1930s, and the involvement of the hypothalamo-hypophyseal portal system summarised in both his *Neural Control of the Pituitary Gland* (Harris, 1955) and masterly Upjohn Lecture of the Endocrine Society (Harris, 1964).

Hysterectomy in laboratory and large farm animals – but not in women or other primates – had indicated that the uterus in some manner influenced the

Table I.1. *Selected volumes concerned with ovarian function in mammals published during the twentieth century*

Marshall (1910)	*The Physiology of Reproduction*
Parkes (1929)	*The Internal Secretions of the Ovary*
Hartman (1936)	*Time of Ovulation in Women*
Pincus (1936)	*The Eggs of Mammals*
Corner (1942)	*The Hormones in Human Reproduction*
Parkes (1956)	*Marshall's Physiology of Reproduction*, 3rd edition
Villee (1961)	*Control of Ovulation*
Young (1961)	*Sex and Internal Secretions*, 3rd edition
Zuckerman & Mandl (1962)	*The Ovary*
Perry (1971)	*The Ovarian Cycle of Mammals*
Austin & Short (1972)	*Reproduction in Mammals*
Greep (1973)	*American Handbook of Physiology*
Mossman & Duke (1973)	*Comparative Morphology of the Mammalian Ovary*
Crosignani & Mishell (1976)	*Ovulation in the Human*
Zuckerman & Weir (1977)	*The Ovary*, 2nd edition
Jones (1978)	*The Vertebrate Ovary*
Midgley & Sadler (1979)	*Ovarian Follicular Development and Function*
Thibault & Levasseur (1979)	*La Fonction Ovarienne chez les Mammifères*
Edwards (1980)	*Conception in the Human Female*
Motta & Hafez (1980)	*Biology of the Ovary*
Peters & McNatty (1980)	*The Ovary. A Correlation of Structure and Function in Mammals*
Austin & Short (1982)	*Reproduction in Mammals*, 2nd edition
Lamming (1984)	*Marshall's Physiology of Reproduction*, 4th edition
Knobil & Neill (1988)	*The Physiology of Reproduction*
Hillier (1992)	*Gonadal Development and Function*
Adashi & Leung (1993)	*The Ovary*
Thibault, Levasseur & Hunter (1993)	*Reproduction in Mammals and Man*
Findlay (1994)	*Molecular Biology of the Female Reproductive System*
Knobil & Neill (1994)	*The Physiology of Reproduction*, 2nd edition
Grudzinskas & Yovich (1995)	*Gametes – The Oocyte*

lifespan of the cyclic corpus luteum. Many experimental approaches were used to unravel the humoral interaction between uterine and luteal tissues, both in cyclic and in pregnant animals. By the end of the 1960s, it was appreciated that luteotrophic (embryonic) and luteolytic (uterine) factors were at play and that it was the balance between these positive and negative factors that determined luteal lifespan. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was proposed as the luteolytic factor by the late 1960s (Pharriss & Wyngarden, 1969), and demonstrated

to be elaborated by the endometrium of a healthy, non-gravid uterus (i.e. in the absence of pyometritis) shortly before the demise of the corpus luteum (McCracken, 1971; Goding, 1974). As to the embryo and working initially with the much elongated conceptus of ungulates (Moor & Rowson, 1964; Rowson & Moor, 1966, 1967), the luteotrophic factor that acts to suppress secretion of uterine $PGF_{2\alpha}$ was shown to be a protein initially termed trophoblastin, a useful name giving some indication of its origin (Martal *et al.*, 1979). However, now that the molecular age is well and truly upon us, the luteotrophic factor – at least in ruminants – is referred to as interferon tau (τ) (Roberts *et al.*, 1999; Winkelman *et al.*, 1999). As a postscript to this paragraph, it should be noted that the ovary itself can also synthesise prostaglandins, both in Graafian follicles and in the mature corpus luteum, at least in primates. Indeed, the latter doubtless affords a means of explaining ovarian cyclicity after hysterectomy in women.

By the 1980s, the hypothalamic-posterior pituitary peptide oxytocin was also shown to be an ovarian hormone (see Flint & Sheldrick, 1982; Wathes & Swann, 1982; Wathes, 1989; Wathes & Denning-Kendall, 1992), and much involved in the events of luteolysis and seemingly more widely in ovarian physiology. And, by the mid-to-late 1980s, many other peptides representing the diverse family of growth factors were shown to be critically involved in ovarian function. Finally, in this brief perspective, reference must be made to the traffic in white blood cells, especially polymorphonuclear leucocytes, through ovarian tissues. Although an observation of long-standing, this field received a particular emphasis during the 1990s, and products of ovarian macrophages such as cytokines are now believed to make a pivotal contribution to tissue modifications close to the time of ovulation (see Brännström, 1997).

A notable feature of ovarian research and indeed of reproductive research in the twentieth century, very much during its second half, was the appearance of major studies not only from Europe and the whole of North America, but also from Japan, the newly founded Israel, Australia and New Zealand, Latin America, India and – in the past twenty years – from China and Korea. In proportion to its size and population, published work from the former Soviet Union remains disappointingly thin. By and large, reproductive research in most of Africa is on an exceedingly modest scale, and this is seemingly true also for much of South-East Asia – Burma, Thailand, Malaysia, Indonesia and Vietnam. And, as noted earlier in this chapter, whereas great endeavours came from ancient Greece and Alexandria 2000 or more years ago, modern contributions from Egypt, Greece and Turkey and indeed most of the Middle East remain slight. Despite the widely trumpeted rôle of the internet, there is no reason to suppose that this situation will change rapidly in the new century.

Good research requires not only modern technology – it also requires initiative, originality, enthusiasm, organisation and hard work, all in ample measure and set against an appropriate social backcloth.

Prospects for the current century

As is widely recognised by most of those immersed in the scientific endeavour, the rate of change, of important discovery, of technical development is not only impressive, it is fast rendering many of the more industrious participants breathless or even exhausted. There is much to be said in favour of bouts of intensive and fruitful research activity but there is also a pressing and absolute requirement for time to read widely, to ponder deeply and to discuss. Daily routines should not simply be composed of the laboratory bench, the operating theatre, the ubiquitous computer screen, and yet all too many colleagues are seemingly hypnotised by the last and strait-jacketed by the first.

Taking time to gaze into the crystal ball – not necessarily a worthy activity – the future for ovarian and ovarian-related research would seem to be full of exciting prospects. For example, the contribution of the autonomic nervous system to normal and abnormal ovarian function certainly needs to be revisited, and significant advances seem possible in clarifying the aetiology of polycystic ovarian disease, to cite just one clinical problem. Molecular studies will enable identification of increasing numbers of genes involved in ovarian function and malfunction, and their downstream products may then become open to manipulation in a precise manner. Even so, it is perhaps the related or dependent reproductive technologies that will contribute most impressively. Here, one could cite, for example, culture and growth of primordial follicles to provide a means of transplantation therapy for aplastic ovaries; isolation and proliferation of appropriate stem cells, whether from cloned or conventional *in vitro*-generated embryos, again to offer a therapeutic approach to defective gonads; cryopreservation of portions of ovarian tissue during irradiation treatment for cancer to offer the prospect of restoring ovarian function after autotransplantation; and targeted tissue 'messengers' for mounting a local attack on ovarian cancer cells. Also to be predicted would be a controlled stimulation of Graafian follicles for purposes of *in vitro* fertilisation without the risks associated with extensive superovulation. And, likewise, a simple means of recognising the time of ovulation without recourse to repeated ultrasonic scanning. Reducing the rate of atresia in ovarian follicular populations should not be beyond the bounds of possibility, thereby offering an attractive route to extending the reproductive lifespan.

Developments in all these and in numerous other reproductive spheres should make the next 20 years a period of unparalleled progress. Thereafter who knows and who would dare to suggest? Not this author. But, taking human nature into consideration and a certain predisposition for civilisations mature in years to court disaster, issues reproductive might well have a dramatically modified constitution or be getting seriously out of hand.

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